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A Halotolerant Mutant of *Saccharomyces cerevisiae*

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FRD, a nuclear and dominant spontaneous mutant of *Saccharomyces cerevisiae* capable of growing in up to 2 M NaCl, was isolated. Compared with parental cells, the mutant cells have a lower intracellular Na^+/K^+ ratio, shorter generation times in the presence of 1 M NaCl, and alterations in gene expression.

Increases in extracellular osmotic pressure elicit a complex response in *Saccharomyces cerevisiae* which involves K^+ uptake (7, 13), induction of the transport system for efflux of toxic Na^+ (8, 12), decreased glycerol permeability and increased glycerol synthesis (1, 3), and increased synthesis of membrane stabilizer trehalose (29), as well as changes in gene expression (19, 28). Yeast cells sense osmostress by at least two different pathways: one is a turgor pressure mechanosensitive channel at the plasma membrane (11); the second involves a protein kinase cascade (4, 5, 17, 18) connected to a two-component, *SS1-SSk1* signal transduction system (18). The osmosensitivity trait in yeast cells has a complex phenotype, since it has been observed in membrane ATPase mutants (20), vacuolar mutants (2, 14, 15), cytoskeletal mutants (23), nonsense suppressors (27), phosphatase 2B (calcineurin) mutants (25), and *PKC1* gene mutants (16, 25, 26). The screening of yeast cells transformed with genomic DNA cloned into a multicopy vector for halotolerance led to the isolation of genes *HAL1* (9), *HAL2* (10), and *HAL3* (6). We describe here some genetic and phenotypic properties of an *S. cerevisiae* mutant hyperresistant to NaCl.

The following *S. cerevisiae* strains were used: A364A (*MAT***a** *gal1 ade1-2 ura1 lys2 tyr1*; ATCC 22244); A7A (*MAT*α *gal1 ade1-2 ura3 his7 lys2 tyr1*); FRD, a 2 M NaCl-resistant mutant which was obtained in this work and which is isogenic to A364A; and FRD (*MAT*α), which is isogenic to FRD (*MAT***a**) and which was obtained in this work by meiotic recombination of diploid FRD/A7A.

Strain A364A in liquid SD medium (0.67% yeast nitrogen base without amino acids, 20 μg of each of the strain nutritional requirements per ml, and 2% glucose) supplemented with 1.0 M NaCl was kept at room temperature with moderate shaking. After 6 weeks, a clone, FRD, capable of growing over solid YPD medium (85% yeast extract, 10% peptone, 2% glucose, and 2% agar) supplemented with 2 M NaCl was isolated. FRD had the same auxotrophic requirements as did its parental strain, A364A. The high-salt-tolerance trait was dominant, and it segregated at 2:2 from 10 FRD/A7A asci (results not shown), demonstrating that the FRD mutation is nuclear and strongly suggesting that it is monogenetic. To the best of our knowledge, this is the first halotolerant mutant of *S. cerevisiae* to be reported.

The high-salt-tolerance character of the FRD cells is shown in Table 1. In the presence of 1 M and 1.5 M NaCl, the FRD generation times increased only up to about 2 and 3.6 times, respectively, relative to the times in normal SD medium, while under the same culture conditions, the times for the parental strain increased about 4.5 and 9.5 times, respectively. This response is Na^+ specific, because in the presence of 1 M or 1.5 M KCl, no significant differences in growth rates for the FRD mutant and its parental strain were detected, except for a longer lag phase for the former in 1.5 M KCl.

Na^+ and K^+ intracellular concentrations for the parental and FRD strains grown up to the middle of the log phase in liquid SD medium supplemented with 1 M NaCl were measured with Na^+- and K^+-selective electrodes (Radiometer G502Na and F2312K, respectively) and calomel reference electrodes as previously described (9) and by flame photometry (Perkin-Elmer 2380 atomic absorption spectrophotometer). Table 2 shows that parental cells have about 75 mM Na^+ and 112 mM K^+. Interestingly, in the FRD mutant, both cation concentrations are altered, because FRD has only about half as much Na^+ and about 60% more K^+. Consequently, its Na^+/K^+ ratio is about three times smaller than that for parental cells.

FRD and parental cells grown up to the middle of the log phase in SD medium and SD medium supplemented with 1 M NaCl were pulse-labeled for 15 min with 20 μCi of [^{35}S]methionine per ml. From the S30 cell fraction, proteins were extracted (30) and resolved by two-dimensional polyacrylamide gel electrophoresis (PAGE) (24). The autoradiographs in Fig. 1 show evidence of the quantitative and qualitative differences between the parental and FRD cells, even in the absence of NaCl (Fig. 1A and B). The most notable one is a group of three proteins in the parental strain (Fig. 1A, arrow 1) that was absent in FRD (Fig. 1B, arrowhead 1). Proteins 2, 3, and 4 in the parental strain (Fig. 1A, arrows 2, 3, and 4) were also absent in FRD (Fig. 1B, arrowheads 2, 3, and 4). Conversely, proteins 5 and 6 in FRD (Fig. 1B, arrows 5 and 6) were not present in the parental strain (Fig. 1A, arrowheads 5 and 6). The protein patterns of parental and FRD cells adapted to 1 M NaCl are shown in Fig. 1C and D, respectively. Radioactivity was mainly incorporated into nine proteins, indicating that their expression under high-salt conditions is favored.

The steady-state protein patterns of the FRD mutant and its parental strain, as revealed with Coomassie blue, are shown in Fig. 2. Several categories of proteins were detected. First, there are the proteins present in the parental strain only both in the absence and presence of 1 M salt (compare arrow 1 in Fig. 2A and C with arrowhead 1 in Fig. 2B and D). Second, there are the proteins present in FRD cells only (compare arrow 2 in Fig. 2B and D with arrowhead 2 in Fig. 2A and C). Third, there are the proteins faintly stained in the absence of salt whose concentrations increase, strikingly, in the presence of salt (compare arrow 3 in Fig. 2A and B with arrowhead 3 in Fig. 2C

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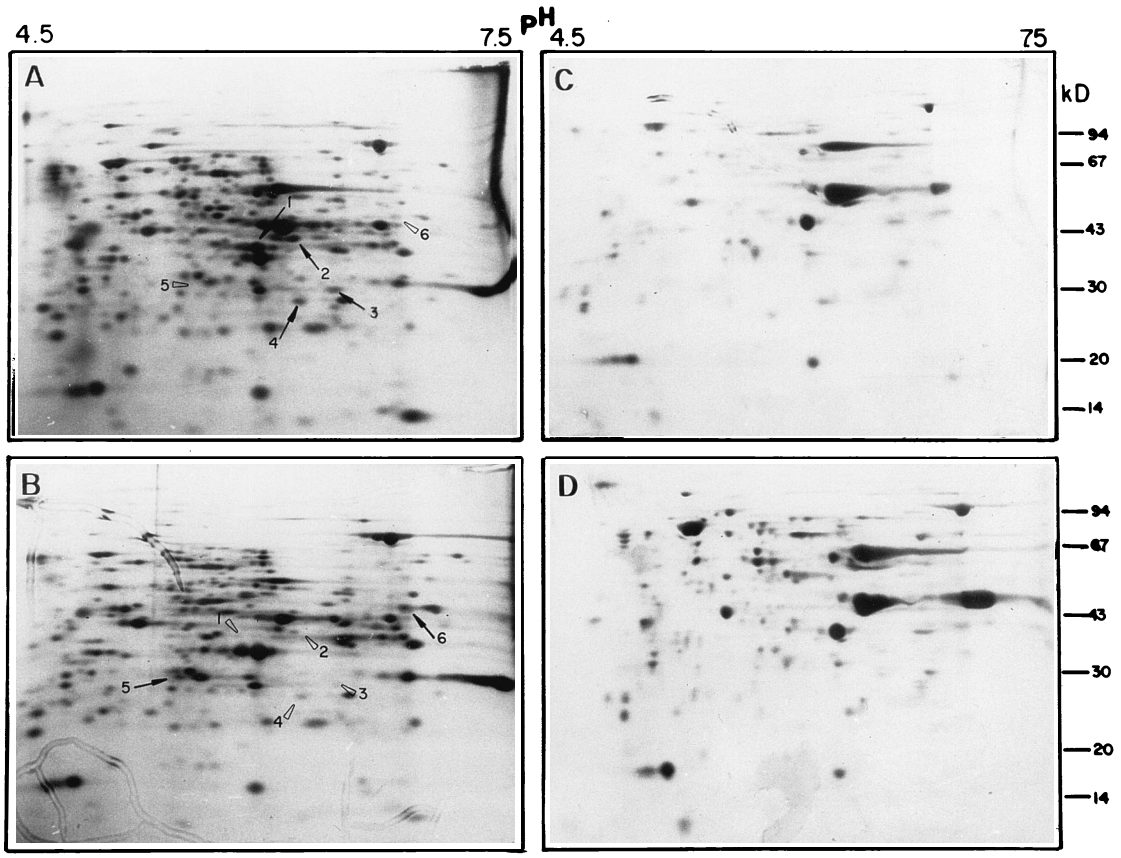

FIG. 1. Autoradiographs of radioactively labeled proteins resolved by two-dimensional PAGE. Parental (A and C) and FRD (B and D) cells growing exponentially in SD medium (A and B) or SD medium supplemented with 1 M NaCl (C and D) were pulse-labeled with [^{35}S]methionine. Arrows and arrowheads with the same numbers point to regions where qualitative or quantitative differences in protein patterns were detected (see the text for a detailed description). Molecular masses are given in kilodaltons on the right.

and D). Fourth, there are the proteins clearly present in FRD cells both in the presence and absence of salt. These proteins were faintly stained in parental cells without salt, and their concentrations increase after salt treatment of parental cells (compare arrow 4 in Fig. 2B and D with arrowhead 4 in Fig. 2A and C). Finally, there are the proteins clearly present in parental cells whose concentrations are notably diminished in FRD cells both in the absence and presence of salt (compare arrow 5 in Fig. 2A and C with arrowhead 5 in Fig. 2B and D). One of the constitutively expressed proteins which is NaCl inducible in the parental strain (Fig. 2, arrow 2) is most probably FRD specific. Altogether, our results indicate that the FRD mutation probably affects more than one level of protein expression.

The most interesting feature of the FRD mutant is its pleiotropic nature, as demonstrated by its high halotolerance (Table 1), its capacity to maintain relatively low Na^+ and high K^+ intracellular concentrations (Table 2), its relatively higher polysomal stability (results not shown), as well as its constitutive and salt-dependent changes in protein expression (Fig. 1 and 2). Since these phenotypic alterations resulted from a single nuclear dominant mutation, they should be explained, therefore, as a change, most probably, in only one *cis*-acting or *trans*-acting regulatory element. Possible direct or indirect targets could be the *HAL3* and/or calcineurin genes (6, 21, 22), the transmembrane osmosensor (5), or a yet unknown element. Mutants lacking functional Hal3p or functional cal-

TABLE 1. Growth parameters of control and mutant cells at various salt concentrations

Salt and strain	Time (h) to cell duplication at salt concn (M) of:			Time (h) to lag phase at salt concn (M) of:		
	0	1.0	1.5	0	1.0	1.5
NaCl						
A364A	1.8	8.0	17.3	5.0	22	24
FRD	2.2	4.2	8.0	6.0	16	42
KCl						
A364A		3.0	5.3			23
FRD		3.0	5.3		13	32

TABLE 2. Intracellular concentrations of cations

Expt and strain	Na^+ concn (mM)	K^+ concn (mM)
1		
A364A	70	115
FRD	36	183
2		
A364A	85	110
FRD	40	190

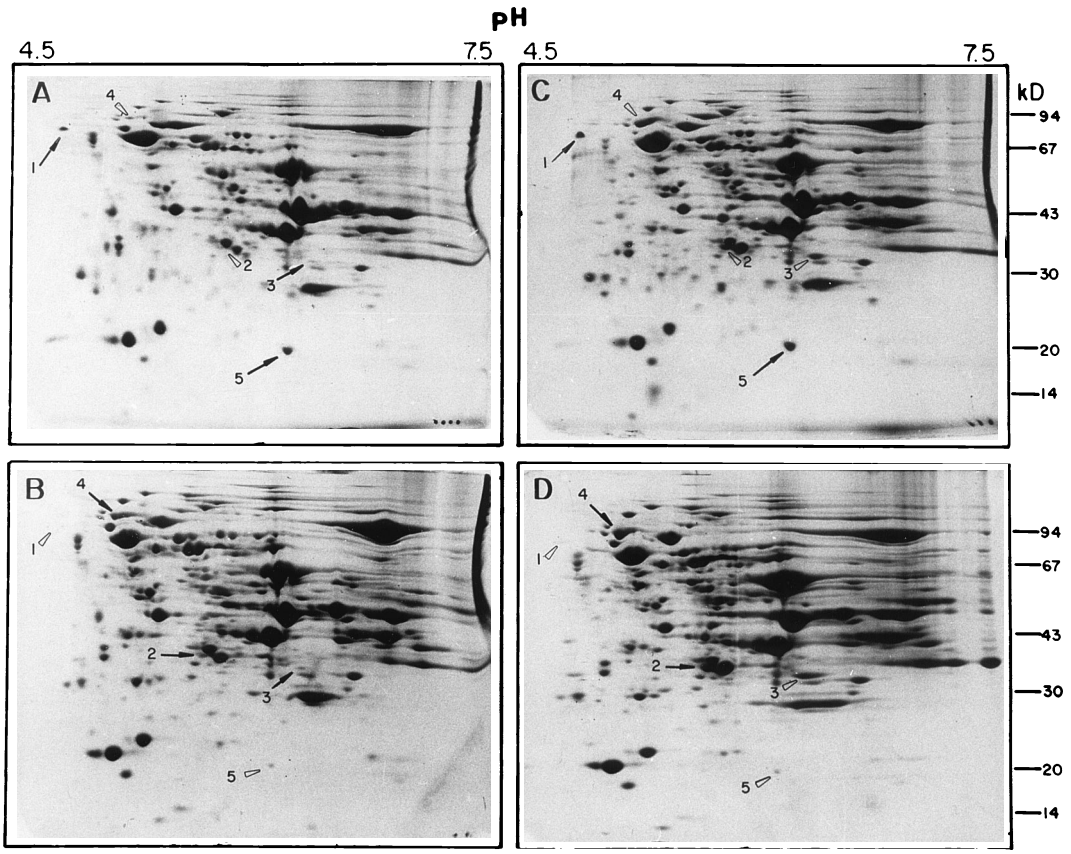

FIG. 2. Coomassie blue-stained protein patterns. Proteins from parental (A and C) and FRD (B and D) cells grown in SD medium without (A and B) and with (C and D) 1 M NaCl were resolved by two-dimensional PAGE (24). Arrows in panels A and C and arrowheads in panels B and D (and vice versa) with the same numbers point to regions where qualitative or quantitative differences in the protein patterns were detected (see the text for a detailed description). Molecular masses are given in kilodaltons on the right.

cineurin have decreased induction of *ENA1/PMR2A* (6), which encodes the system for efficient Na^+ transport (8, 12). In addition, Hal3p activates K^+ uptake during salt stress (6), and calcineurin increases the affinity of the *TRK1,2* import system for K^+ and Na^+ (21, 22). Such combined effects on both cations would explain the low Na^+/K^+ ratio detected in FRD cells (Table 2). A low Na^+/K^+ ratio could result in higher stability for polysomes and differences in protein expression.

An effort to identify the mutated locus in FRD cells is now in progress.

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